COMPARISON OF LEAD-TOLERANCE AMONG RHIZOSPHERIC FILAMENTOUS FUNGI ISOLATED FROM A POLLUTED SITE.

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Introduction. Survival of fungi in presence of heavy metals depends on their biochemical, structural and physiological characteristics, that define the degree of tolerance to heavy metal exposure. Some fungi secrete chelating compunds that sequester metal ion extracelullary, others actively transported metal ion into the cell. In addition, fungal cell walls bind significant amounts of metal ions, and melanins and fungal pigments are related with the endurance in response to environmental stress[(1)]. In Avalos, Chihuahua, the soil contains high levels of Pb (5400 $mg \cdot kg^{-1}$ soil)[(2)]. Rhizospheric fungal strains from this place have been isolated and identified, however until now, their tolerance to lead is unknown.
The aim of this work was to compare the lead tolerance of three rhizospheric filamentous fungi.

Methods. PDA plates supplemented with $Pb(NO_3)_2$ to reach 0, 250, 1000 and 1500 mg/L of Pb^{2+} were prepared. Controls with $NaNO_3$ was used to compensate the nitrate supplied in $Pb(NO_3)_2$.Three replicates of each concentration and controls were used. Fungi strains isolated from Avalos, Chihuahua *(Aspergillus sp., Cunninghamella sp. and Mycotypha sp.*) were needle stick-inoculated. Diameter mycelial growth was taken daily for 15 days, by triplicated. The percentage of inhibition (PI) was determined by the following equation [(3)]:

$$PI = \left(1 - \frac{Pb\ exposure\ \phi}{control\ \phi}\right) \times 100$$

Results. All fungi assayed were able to grow at all the Pb concentrations tested (Table 1). The PIs of all fungi increased as the amount of Pb. *Aspergillus sp.* showed lower PIs than the other fungi, except in 250 ppm. PIs of *Aspergillus sp.* were similar regardless the concentration of Pb. This genera is well known for its high bioadsorption capacity and showed be able to growth until 4000-5000 ppm de Pb^{2+}[(4)].Growth of *Cunninghamella sp.* was weakly inhibited in presence of 250 ppm of Pb, whereas it was the less tolerant at 1500 ppm. *Mycotypha sp.* was inhibited faster than the other fungi and is the most sensitive at low Pb concentrations. The growth of *Mycotypha* on the medium with Pb was accompanied with the secretion of a dark pigment that diffuses into the agar and surrounded the colonies (Fig. 2).

Table 1. Percentage of inhibition after 120 hours of inoculation

mg $Pb \cdot L^{-1}$	Percentage of inhibition (%)		
	Aspergillus sp.	*Mycotypha sp.*	*Cunninghamella sp.*
0	0.0	0	0
250	19.7	50.5	16.9
1000	25.2	68.4	34.2
1500	29.8	78.8	88.3

In other fungi, such as *Verticillium dahliae* and *Phomopsis spp.* melanin was associated with electron-dense granules present in a fibrillar matrix that extends outward from the cell [(5)]. This granules may be released into the external medium, as apparently occurred in *Mycothypha* as response to the stress by the presence of Pb, and may be constitute a mechanism that enabled the fungus grew in Pb.

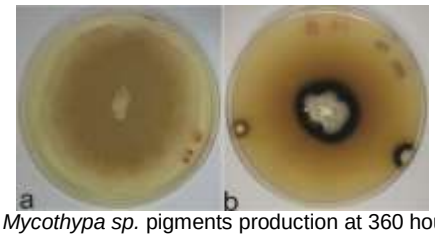

Fig.1 *Mycothypa sp.* pigments production at 360 hours-old cultures. (a) 0 ppm Pb plate (b) 500 ppm Pb plate

Conclusions. *Aspergillus sp.* was the most tolerant fungus among the assessed fungi. Tolerance mechanism in *Mycotypha sp.* may be involved with production of pigments.

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